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Development of Genetic Markers for Environmental DNA (eDNA) Monitoring of Sturgeon

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EXECUTIVE SUMMARY: All living organisms shed DNA into the environment. Recent studies have demonstrated that environmental water samples can be effectively assayed for the DNA of target organisms. The DNA contained in these samples is commonly referred to as environmental DNA or eDNA. Because DNA assays can be sensitive to very small amounts of genetic material, eDNA surveys have the potential to detect sites where target organisms occur at very low abundances, potentially even when organisms are too rare for conventional survey methods to effectively detect them. We designed and tested twelve new eDNA markers for aquatic eDNA surveys of North American sturgeon. Eight of the markers are general for all North American sturgeon (i.e., not genus- or species-specific), two are specific to *Scaphirhynchus* sturgeon, and two are specific to white sturgeon (*Acipenser transmontanus*). The *Scaphirhynchus* marker could identify sites where endangered species like the pallid (*S. albus*) or Alabama (*S. suttkusi*) sturgeon might occur. Positive detections with the general sturgeon marker combined with the absence of positive detections with the white sturgeon marker could be used to discern if green sturgeon (*A. medirostris*) might occur at a location. All sturgeon markers were tested for specificity against a battery of 32 non-target fish species common to the Mississippi and Illinois River watersheds and the "sensitivity" or limit of detection for each marker was determined with assays of increasingly dilute solutions of target DNA. Four of the general sturgeon markers were used to assay 88 water samples from the Lower Mississippi River for sturgeon eDNA and resulted in 3 positive detections. While additional optimization of sturgeon-specific sampling protocols and marker assays is advised prior to use of these markers for sturgeon eDNA surveys, the newly designed markers represent a significant step forward in the use of eDNA in sturgeon monitoring and management.

PURPOSE: This technical note focuses on the development of environmental DNA (eDNA) markers for detection of North American sturgeon species. Topics include design and laboratory testing of markers for a polymerase chain reaction (PCR)-based eDNA assay and an overview of good laboratory practices for the handling and processing of eDNA samples.

INTRODUCTION: Many aquatic species are difficult to detect and accurately monitor using conventional sampling techniques. Such methods, including fishing, netting, seining, and electrofishing, can often be logistically complex and require considerable outlays of time and funding, without accurately reflecting presence or abundance of target species (Portt et al. 2006). This is particularly true for rare or elusive species. eDNA provides an alternative means for detecting and monitoring aquatic species. Rather than relying on physical capture of an organism, procurement of water samples containing trace genetic evidence of a species' presence can be used

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to detect target species and estimate their abundance (Ficetola et al. 2008, Lodge et al. 2012, Takahara et al. 2012). Such an approach is possible because all aquatic species release DNA into the environment in the form of somatic and gametic cells that are shed via natural sloughing of epithelia (including cells shed through urination and defecation), injury or predation, and reproduction. Recently, eDNA assays have been used to monitor invasive species such as Asian carp (Jerde et al. 2011), zebra mussels (Lance and Carr 2012), and bullfrogs (Ficetola et al. 2008), as well as to detect endangered species of amphibians (Goldberg et al. 2011), and to determine the presence of a broad range of other aquatic and semi-aquatic species (Thomsen et al. 2012). Although typically used for species presence/absence information, eDNA methods are rapidly being expanded to provide information on fish and amphibian community composition (Minamoto et al. 2012, Thomsen et al. 2012) and biomass estimation (Takahara et al. 2012).

Sturgeon are taxa for which eDNA-based monitoring approaches could be particularly helpful. Because they are bottom-dwellers, and because they are often found at low abundance in large bodies of water, sturgeon can be difficult to sample by traditional methods (LeBreton et al. 2004). Many sturgeon species are also anadromous, migrating up and down waterways during their life cycle, resulting in large seasonal shifts in distribution and habitat usage (Bemis and Kynard 1997). The majority of sturgeon species in North America are protected under the Endangered Species Act, requiring the US Army Corps of Engineers (USACE) and other agencies to carefully monitor sturgeon populations and plan work projects to minimize impacts on these species. An eDNA assay for sturgeon could provide a more logistically feasible and cost-effective strategy for monitoring these species.

PCR is a laboratory reaction used to enzymatically increase the abundance of a specific target DNA sequence in solution. PCR is based on a series of heating and cooling steps that modify the activity of a DNA copying enzyme (or DNA polymerase). The DNA sequence of interest, the marker, is targeted using "primers," which are short fragments of DNA that bind to both ends of the target sequence. Primers serve, essentially, as starting and stopping points for the DNA polymerase. In the initial step of a PCR, the reaction volume is rapidly heated, causing the two strands of the double helix to separate (i.e., denaturation). The reaction is then rapidly cooled, at which point the primers bind to the ends of the target sequence on both strands. The PCR volume is then heated to an intermediate temperature (well below the level of denaturation) at which the primers can serve as essential starting points for DNA polymerases to copy the original DNA strands. After a short period, the DNA solution is again heated to the level of strand denaturation and the PCR cycling process starts over, this time with both the original template DNA and the initial PCR copies serving as templates for the next round of copying (or amplification). This cycling process, repeated many times (e.g. 30-40 cycles), results in a large increase in the number of DNA copies and includes a range of cycles over which exponential increases in copy number may occur. In the end, the PCR solution may hold billions of copies of the marker, which can then be used in additional laboratory procedures such as gel electrophoresis and DNA sequencing. Because eDNA assays are based on PCRs of environmental sample templates with DNA from many different organisms, the key to effective eDNA assays is the exclusivity of the PCR marker (derived largely from primer specificity) and protocols.

Ideal primers and protocols are accurate (amplify only the species of interest), sensitive (detect very small amounts of DNA in solution), and robust (perform reliably despite variation in water

chemistry among sites, time points, etc.). Generally, eDNA markers (targeted DNA sequences) are selected from among species-unique regions of the mitochondrial DNA (as opposed to the nuclear DNA). Only a few copies of a nuclear marker occur per cell in a typical diploid organism, while there can be many copies of mitochondrial DNA (mtDNA) in each cell (thousands of copies in some cases). Therefore, the probability of detecting an mtDNA marker should be significantly higher than for a nuclear DNA marker. Additionally, marker selection should favor DNA sequences that are short. When genetic material is released into the environment, DNA molecules begin to break down, from entire chromosomal strands into smaller and smaller fragments over time. Because shorter DNA markers are more likely to remain intact over time in environmental media, and because PCR-based detection requires that the entire DNA marker segment be intact, shorter markers provide a greater likelihood of detection (Sefc et al. 2003).

MATERIALS AND METHODS

Development of general markers for detection of sturgeon eDNA. Markers for the general detection of Acipenseridae were designed using complete mitochondrial genome sequences that were already available for seven sturgeon species in the National Center for Biotechnology Information (NCBI) GenBank database. These sequences include two North American species, the white (*Acipenser transmontanus*) and pallid (*Scaphirhynchus albus*) sturgeon, and five Eurasian species (*A. dabryanus, A. gueldenstaedtii, A. sinensis, A. stellatus,* and *Huso huso*). ClustalW (Larkin et al. 2007) was used to align these sequences along with sequences from paddlefish (*Polyodon spathula*) and largemouth bass (*Micropterus salmoides*). GenBank accession numbers for all nine species sequences used are AB042837, AP004354, AY510085, FJ392605, EU719645, AJ585050, AY442351, AP004353, and NC_008106, respectively. Paddlefish are the closest extant evolutionary and genetic relatives to sturgeon. Therefore, eDNA markers that can differentiate between sturgeon and paddlefish are likely to be selective for sturgeon against all other species. Largemouth bass were included in the alignment as a more distantly related outgroup. Sequence regions that were conserved among sturgeon species, but differed significantly from paddlefish and bass, were identified as possible primer sites for eDNA markers. DNA sequences that would result in eDNA markers in a size range of 100-300 base pairs were targeted and input into Primer3 software (Rozen and Skaletsky 2000) to aid primer development and optimize compatibility for each primer pair. DNA sequences for potential primer sites were compared to all DNA sequence data found in GenBank to minimize the likelihood that the PCR assay would amplify DNA sequences from non-sturgeon species.

Each potential set of primers, or markers, was tested for successful PCR amplification against single genetic samples from each of the nine North American sturgeon species (Table 1). Using DNA extracted from various tissues, PCR trials for each marker and each species were conducted under the following conditions: 25-µL total volume reactions containing 2.5 µL of 10x Buffer solution, 0.75 µL 50 mM $MgCl_2$, 0.5 µL 10 mM dNTP mixture, 0.5 µL of each primer (10 µM each), 0.5 units Platinum *Taq* polymerase, and 1 µL of DNA template. The thermal-cycling protocol included an initial melting step at 94 °C for 2 minutes, followed by 40 amplification cycles (94 °C for 30 seconds, annealing temp for 15 seconds, 72 °C for 30 seconds). In order to determine the optimal temperature for each marker assay across all nine species, initial PCRs were conducted across a gradient of annealing temperatures ranging from

48-62 °C. Gel electrophoresis runs of resulting PCR products were performed on a 1.5% ethidium bromide gel and visualized under UV light.

Table 1. North American Sturgeon and Their Status Under the US Endangered Species Act

Common Name	Scientific Name	Conservation Status
Atlantic sturgeon	*Acipenser oxyrinchus oxyrinchus*	E (4 ps), T(1 ps)
Gulf sturgeon	*Acipenser oxyrinchus desotoi*	E
Shovelnose sturgeon	*Scaphirhynchus platorynchus*	NL
Pallid sturgeon	*Scaphirhynchus albus*	E
Lake sturgeon	*Acipenser fulvescens*	NL
Alabama sturgeon	*Scaphirhynchus suttkusi*	E
Shortnose sturgeon	*Acipenser brevirostrum*	E
Green sturgeon	*Acipenser medirostris*	T (1 ps), SC (1 ps)
White sturgeon	*Acipenser transmontanus*	E (1 ps), NL

E = Listed as "endangered" under United States Endangered Species Act (ESA)
T = Listed as "threatened" under ESA
ps = Distinct population segment as defined for ESA (61 FR 4722; February 7, 1996)
NL = Not listed under ESA
SC = Listed as Species of Concern under ESA

Development of genus- and species-level eDNA detection tools. While the primary focus of this project was the development of family-level eDNA markers for surveying sturgeon, eDNA markers for targeting particular sturgeon species are probably of even greater interest. The use of "blocking primers" in tandem with the new general Acipenseridae markers that had been designed was explored as a means for detecting one particular species of sturgeon where two or more species co-occur. Blocking primers are short pieces of DNA designed to have a high affinity (or binding potential) for the DNA sequence(s) of particular taxa and a low affinity for competing DNA sequences from other taxa. In PCR reactions, these blockers attach to the DNA of the species for which no amplification is desired, while allowing the amplification of DNA from desired (or target) species (Terahara et al. 2011). As an alternative to relying on the combination of general sturgeon markers and blocking primers, additional primers were designed to provide markers for detecting 1) only *Scaphirhynchus* sturgeon DNA, and 2) only *A. transmontanus* DNA. Combining general sturgeon marker assays with assays using taxa-specific blocking primers or markers should allow for more fine-tuned surveys and monitoring. For example, either by themselves, or in combination with general sturgeon markers, *Scaphirhynchus*-level markers would allow managers and scientists to identify locations where endangered *S. albus* or *S. suttkusi* occur. This might be particularly useful in watersheds where *S. suttkusi* historically occurred, but where the much more common *S. platorynchus* is not found. Likewise, a combination of general sturgeon markers with *A. transmontanus*-level markers might allow managers and scientists to determine whether sturgeon eDNA detected at sites within the broadly overlapping ranges of *A. transmontanus* and *A. medirostris* comes from either or both species.

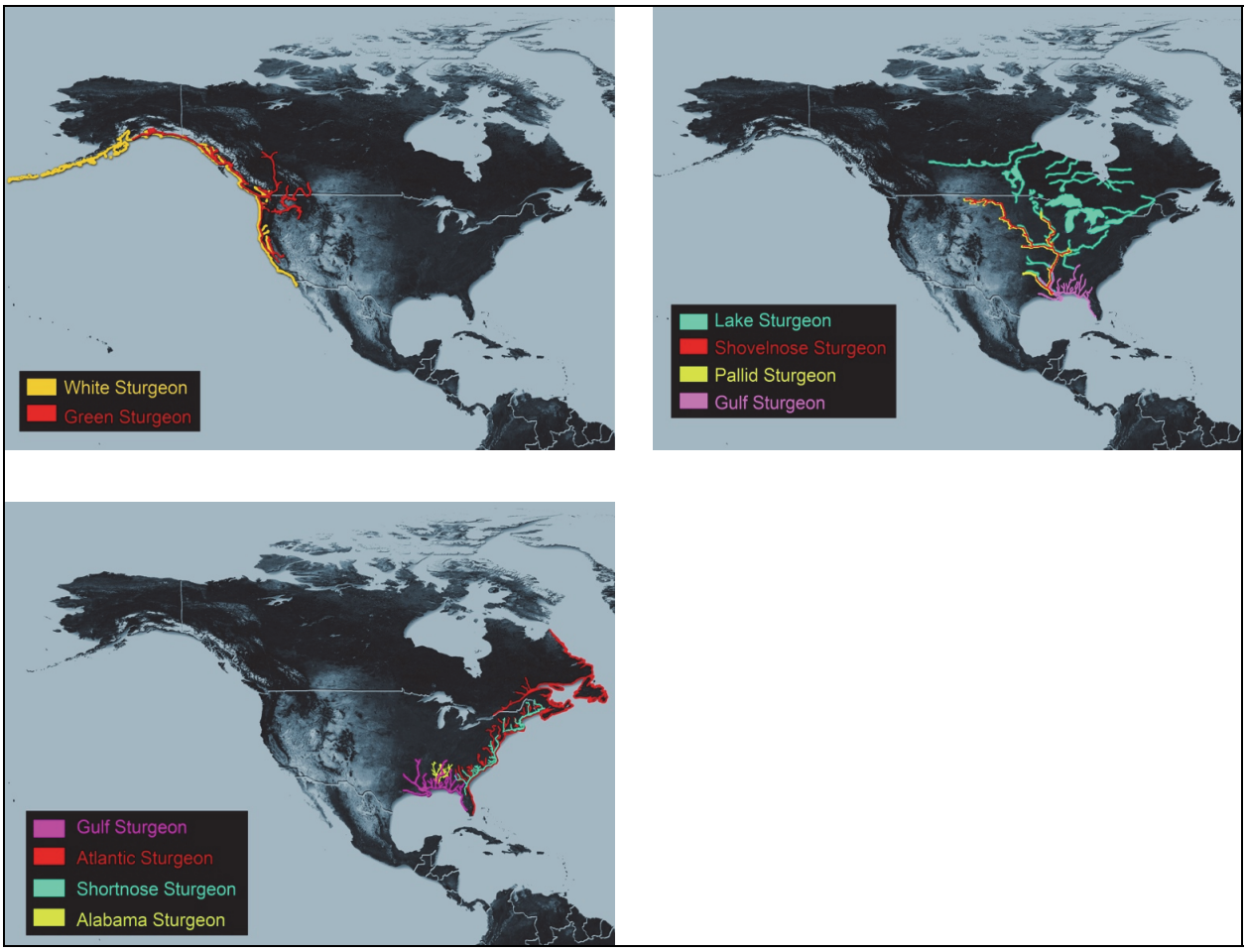

Figure 1. Overlaps in ranges of nine North American sturgeon species and subspecies.

Marker laboratory testing. Following PCR optimization, each marker was further tested for cross-taxa amplification (or selectivity) using DNA from a battery of 32 fish species commonly found in the eastern/central United States (Table 2). These trials, intended as secondary validation of selectivity findings from GenBank sequence comparisons, assessed, to some degree, the potential for undescribed DNA sequences (allelic variants, pseudogenes, numts, etc.) in non-target species to produce false positive results (Moulton et al. 2010). Finally, the sensitivity (lowest amounts of DNA detected) for each marker was determined by PCR trials with increasingly dilute concentrations of sturgeon genomic DNA.

Table 2. Non-sturgeon Species Used in eDNA Marker Specificity Testing.	
Common Name	**Scientific Name**
Brown bullhead	*Ameiurus nebulosus*
Freshwater drum	*Aplodinotus grunniens*
Goldfish	*Carassius auratus*
Quillback	*Carpiodes cyprinus*
Grass carp	*Ctenopharyngodon idella*
Spotfin shiner	*Cyprinella spiloptera*
Common carp	*Cyprinus carpio*
Mirror carp	*Cyprinus carpio carpio*
Gizzard shad	*Dorosoma cepedianum*
Silver carp	*Hypophthalmichthys molitrix*
Bighead carp	*Hypophthalmichthys nobilis*
Smallmouth buffalo	*Ictiobus bubalus*
Black buffalo	*Ictiobus niger*
Channel catfish	*Ictalurus punctatus*
Brook silverside	*Labidesthes sicculus*
Green sunfish	*Lepomis cyanellus*
Pumpkinseed sunfish	*Lepomis gibbosus*
Orange-spotted sunfish	*Lepomis humilis*
Bluegill	*Lepomis macrochirus*
Smallmouth bass	*Micropterus dolomieu*
Largemouth bass	*Micropterus salmoides*
White perch	*Morone americana*
White bass	*Morone chrysops*
Round goby	*Neogobius melanostomus*
Golden shiner	*Notemigonus crysoleucas*
Emerald shiner	*Notropis atherinoides*
Yellow perch	*Perca flavescens*
Bluntnose minnow	*Pimephales notatus*
Paddlefish	*Polyodon spathula*
White crappie	*Pomoxis annularis*
Black crappie	*Pomoxis nigromaculatus*
Flathead catfish	*Pylodictis olivaris*

Assay field testing. In addition to laboratory testing, a small field trial with the most promising eDNA markers was conducted. Eighty-eight water samples (44 surface and 44 near-bottom) were collected in early September 2013, from the main channel of the Mississippi River near Vicksburg, MS. These samples were collected for another eDNA study, so methods were somewhat modified from those described in the *A Note on eDNA Quality Assurance and Control* section below. Samples were collected in 50 mL tubes and centrifuged at 4°C for 30 minutes at 3500-4000 rpm. The supernatant was then poured off and DNA was extracted from the remaining pellet material using a modified cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle 1987). Subsequent PCR utilized the same protocols and reagents as those

described above in the Development of General Markers for Detection of Sturgeon eDNA section, with an annealing temperature of 56 C. Additional information related to field trial methods is available upon request from the authors.

Table 3. Marker Information.[1]

Marker	Primer	Primer Sequence (5'-3')	Location	Gene	T_m	PCR Product (bp)
General Sturgeon Markers						
StrG1	F	GTACACGGACTATGAAGACCTG	7556	Cox2	56	297
	R	GGCATGAAGCTGTGGTTAG	7834		57	
StrG2*	F	AATCTGAGGCGGCTTTTC	14915	cytB	58	234
	R	GAGGTGAGTCCGACTAGCATT	15128		58	
StrG3	F	ACCTCCAACTTTTTACCAGC	13417	ND5	56	164
	R	AAGTTATGTAGTGGGATTGTGG	13559		56	
StrG4*	F	CTACTAAAACTTGGTGGCTACG	11109	ND4	56	313
	R	TGTGAAGGCGTTCATAGTTAG	11401		57	
StrG5	F	CATCATCGGCTCTACCTTCC	9460	Cox3	60	311
	R	GTAACCAGAATGCAACGACTG	9750		58	
StrG6*	F	CCCAACTGGCATTATCACCT	9187	Cox3	60	276
	R	ATGACATGAAGTCCGTGGAAC	9442		60	
StrG7	F	GCTAGGCCATACCTTCACGAAC	8079	ATP6	62	269
	R	CCGAGGTTGATGGGCAGTA	8329		62	
StrG8*	F	TCCAACGACTTGACTTGTAACC	10439	ND4	59	245
	R	GTTGGCGGCCGATTG	10669		61	
Scaphirhynchus-specific Markers						
StrSc2N	F	CAACAACCCAACAGGACTA	15042	cytB	56	94
	R	CATTAGGATGAACCCTAGTAGATC	15109		55	
StrSc4N	F	TATGACAGGATCGATCTGTTTAC	11217	ND4	56	205
	R	TGTGAAGGCGTTCATAGTTAG	11402		57	
Acipenser transmontanus-specific Markers						
StrAci1N	F	AATGGTAGTTCCCATAGAATCTC	7652	Cox2	56	164
	R	ATTGGCCATAGTAAACTCCTG	7795		56	
StrAci2N	F	GCATAATCCACCTCTTATTCC	15004	cytB	55	87
	R	GGTGGAATGTTACTTTGTCC	15071		54	

[1]Although markers were amplifiable across a wide range of annealing temperatures, laboratory testing was typically performed with annealing temperatures of 56-60 °C.
Location and gene association are based on alignment with the complete mitochondrial genome sequence for *A. transmontanus* (GenBank Accession: AB042837; Inoue et al. 2003) or *Scaphirhynchus albus* (GenBank Accession: AP004354; Inoue et al. 2003) in the case of primers specific for this genus. Gene codes: Cox= cytochrome c oxidase subunit, ND= NADH dehydrogenase subunit, cytB= cytochrome b, ATP= ATP synthase. T_m is the primer melting temperature and used to calculate optimal PCR annealing temperatures. Starred markers are those that performed best in laboratory cross-amplification and sensitivity trials.

RESULTS AND DISCUSSION

Marker laboratory testing. Eight potential eDNA markers (i.e. primer pairs) from within the mtDNA were identified for general use with Acipenseridae (Table 3). Most markers worked well across all North American sturgeon species and at a wide range of annealing temperatures, with the

following exceptions. StrG5 did not work efficiently in the *Scaphirhynchus* genus (shovelnose, pallid, and Alabama sturgeon), yielding multiple products at low annealing temperatures (<54 °C) and no products at higher temperatures (>54 °C). StrG7 required a slightly lower range of annealing temperatures (48-54 °C) for *A. fulvescens* (lake sturgeon) amplification when compared to other markers, and generated multiple products for lake and shovelnose (*S. platorynchus*) sturgeon at lower annealing temperatures (48-52 °C). Markers were typically tested simultaneously in the laboratory and an annealing temperature of 56 °C was chosen as the standard. Therefore, primer sets StrG5 and StrG7 were excluded from further testing due to their required lower annealing temperature.

Tests for marker selectivity against the battery of non-target fish revealed some additional limitations. Detection of non-target fish became an issue when lower annealing temperatures (≤56 °C) or higher cycle numbers (>30) were employed in PCR. Such results are not surprising as lower annealing temperatures decrease primer binding specificity and higher cycle numbers increase the amount of PCR product resulting from rare non-target primer binding events (Roux 2009). For example, no occurrences of cross-species amplification (i.e. detection of nontarget species) occurred with markers StrG2, StrG3, StrG4, and StrG8 at 56 °C and 30 cycles, but raising the number of cycles to 40 drastically increased cross-amplification of nontargets. Marker StrG3 had a tendency to amplify paddlefish DNA at 56 °C, but not at 60 °C for 40 cycles. Marker StrG1 had the highest occurrence of cross-species amplification (including paddlefish and all tested shiners) at 56 °C for 40 cycles. No cross-amplification of nontarget species was observed for markers StrG2, StrG4, StrG6, and StrG8 when an annealing temperature of 60 °C was used, even with as many as 40 PCR cycles. The optimal PCR conditions for eDNA assays are always a balance between annealing temperatures and cycle numbers, or in other words, a balance between maximizing the amplification of even small amounts of target DNA (sensitivity), while limiting the amount of nontarget cross-amplification (inaccuracy). The best approach for each study will depend on the relative values of sensitivity and accuracy.

In general, the markers developed were able to produce visible amplification (bands on agarose gels) for genomic DNA solutions with as little as $3x10^{-4}$ to $3x10^{-5}$ng/μL DNA using a basic PCR protocol with an annealing temperature of 56 °C and 45 cycles. Changes to PCR conditions (*Taq* polymerase and Mg^{++} concentrations) did not significantly alter the sensitivity of the assay.

Assay field testing. Based on the trials described above, markers StrG2, StrG4, StrG6, and StrG8 were the best candidates for use as eDNA markers for sturgeon and were further tested using 88 field samples collected from the Mississippi River. These field tests were largely unproductive, with only three samples testing positive for the presence of sturgeon DNA. One detection occurred with marker StrG6, while the other two detections occurred with StrG8. Each positive test was from a different water sample.

The combination of assaying for sturgeon, which are typically found in lower numbers than many other species for which eDNA sampling has been performed (e.g., silver and bighead carp, bluegill, and bullfrogs) and assaying in the extremely high volume main channel of the Mississippi River may explain why there were only a small number of positive detections. Additionally, the sample volumes obtained for this field trial were relatively small (e.g., 40 times smaller than a typical 2-L sample collected and filtered for eDNA monitoring of Asian carp; Jerde et al. 2011),

though on par with some other studies (15 ml samples utilized in Ficetola et al. 2008 and Thomsen et al. 2012). Sturgeon behavior and habitat use is also considerably different from Asian carp, bluegill, and other species that eDNA has been successfully applied to, and these differences might also affect eDNA detection rates. In any case, whether any of these proposed factors affected the results of this study, additional sturgeon-focused optimization of eDNA sampling methods would be recommended before these markers are employed in surveying for sturgeon eDNA.

Genus- and species-level assays. In the case of North American sturgeon, no DNA sequence variation was apparent between species of *Scaphirhynchus* for the mitochondrial regions from which the eight StrG markers were designed, which made blocker-based species-specific detection impossible. However, there were sufficient genetic differences between *Scaphirhynchus* and *Acipenser*, as well as between several *Acipenser* species, to pursue taxon-specific blocker-based detection. Several peptide nucleic acid (PNA) blocking primers were designed and tested for selective amplification of *Scaphirhynchus* vs. *Acipenser*, which would provide species-specific detection in some regions of North America. Blocking primers were also designed for *A. medirostris* vs. *A. transmontanus*, which co-occur in several watersheds of the western United States. However, in every case in which PCR blockers completely prevented amplification of one taxon, amplification was also dramatically reduced in the target group. Furthermore, in most cases, the blockers did not completely prevent PCR amplification and detection of the non-target species. The poor results from these trials were likely due to limited sequence differences among species and, therefore, insufficient differences in interspecific PNA-DNA template affinities. Additional data on PNA sequences and trials are available from the authors.

Another approach for species-specific eDNA detection in areas with multiple sturgeon species was to design new markers with PCR primers that aligned along mtDNA regions where significant interspecific sequence differences occur. This approach, as with the blocking primer method, is difficult to achieve for closely related species. Of nine new markers designed to detect either *Scaphirhynchus* or *Acipenser*, or to detect either *A. medirostris* or *A. transmontanus* (green and white sturgeon), four performed as expected. These primers and their taxon specificity are also listed in Table 3. Additional testing and development will be required to optimize these markers for field use. Complete mitochondrial genome sequencing of all North American sturgeon, especially the *Scaphirhynchus*, would significantly further efforts to develop species-specific markers.

A note on eDNA quality assurance and control. Current eDNA sample processing procedures used by USACE are modeled after the Asian Carp Monitoring Project in the Chicago Area Waterway System. The Quality Assurance Project Plan (QAPP) for this endeavor details eDNA sample processing procedures and can be found at *http://www.asiancarp.us/documents/USACE-eDNA-QAPP.pdf*. The following is a brief summary of the eDNA assay.

1. Two-liter water samples are collected from the field and returned to a clean lab for vacuum filtration through a 1.5-micron glass fiber filter. Filters are then stored frozen (-20 °C) until sample processing.
2. DNA is extracted from the filter and purified using a commercial DNA extraction kit. The final extract will contain a mixture of DNA from various organisms.

3. Replicate PCR assays are then performed on the eDNA extract using primers designed for the target species.
4. PCR products are run through gel electrophoresis on an agarose gel (e.g. 2%) and visualized under ultraviolet light. Any bands appearing at the target DNA fragment size (presumptive positives) are collected and purified for DNA sequencing.
5. Fragments are DNA sequenced and results are compared to the NCBI GenBank database or a reference sequence to verify identity.

Because eDNA assays are designed to be sensitive to small quantities of DNA, contamination is a serious concern during sample processing. Contamination of equipment, work surfaces, or reagents can introduce genomic DNA or PCR amplicons from the target species into PCR mixes and result in false positives in eDNA samples. Several laboratory practices can be utilized to safeguard against contamination:

1. Physical separation of tasks. Pre- and post-PCR work areas should be isolated to limit the potential for PCR amplicons entering the sample processing areas.
2. Extensive use of negative controls. Controls should include equipment controls, as well as extraction and PCR controls, to test equipment and reagents for contamination.
3. Sterilization of work areas. Ultra-violet irradiation and bleaching of work areas and equipment can destroy or alter any unwanted DNA so that it is no longer a potential contaminant.
4. Special equipment. Laboratory items such as filtered pipet tips or specially designated supplies and storage areas can help contain samples and limit movement and handling of samples and equipment.

Guidelines appropriate for using high-sensitivity PCR with degraded DNA can be obtained from a number of sources, including Gilbert et al. 2005, Hummel 2003, and Shapiro and Hofreiter 2012.

ADDITIONAL INFORMATION: This technical note was prepared by Heather L. Farrington (919-707-9283, *Heather.Farrington@naturalsciences.org*) and Richard F. Lance (601-634-3971, *Richard.F.Lance@usace.army.mil*), Environmental Laboratory, US Army Engineer Research and Development Center. The study was conducted as an activity of the Ecosystem Management and Restoration Research Program (EMRRP). For information on EMRRP, please consult *http://el.erdc.usace.army.mil/emrrp/emrrp.html* or contact the Program Manager, Glenn Rhett, at *Glenn.G.Rhett@erdc.usace.army.mil*. This technical note should be cited as follows:

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